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Bacterial Degradation of the Polycyclic Aromatic Hydrocarbon (PAH) -Fraction of Refinery Effluent

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Abstract The occurrence of polycyclic aromatic hydrocarbons (PAH) in refinery effluents is of great concern globally due to its persistence, recalcitrance and carcinogenicity. This study was aimed at bacterial degradation of polycyclic aromatic hydrocarbons (PAH) in untreated refinery effluent. Contaminated soil samples were collected from Warri refinery jetty while water sample was collected from Ekpan River and untreated effluent was collected from Warri refinery and Petrochemical Company. The total heterotrophic bacterial count ranged from 0.6×10^6 to 2.7×10^6 cfu/ml for soil samples and 3.2×10^6 cfu/ml for water sample. The hydrocarbon utilizing bacteria count ranged from 1.4×10^3 to 2.0×10^3 cfu/ml for soil samples and 1.0×10^3cfu/ml for water sample. Among the 41 bacterial isolates capable of utilizing crude oil vapour, 26 gave a positive emulsification activity on crude oil while only 3 were capable of degrading anthracene and phenanthrene. The three (3) PAH-degraders were characterized and identified as *Pseudomonas*sp(SB), *Achromobacterxylosoxidans*(SB1) and *Pseudomonas*sp(SB3). The percentage degradation of the polycyclic aromatic hydrocarbons in the refinery effluent after 360hours was highest in the mixed bacterial culture with 97.90% and this was followed closely by *Pseudomonas*sp(SB) with 96% and *Achromobacterxylosoxidans*(SB1) having the lowest with 93.40%.

Keywords*: refinery effluent, polycyclic aromatic hydrocarbons, biodegradation, biosurfactant*

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1. Introduction

Petroleum refinery effluents are wastes originating from industries primarily engaged in refining crude oil and manufacturing fuels, lubricants and petrochemical intermediates (Harry, 1995; Saien and Nejati, 2007). The high polycyclic aromatic hydrocarbon content of refinery effluent is an issue of environmental concern owing to the fact that polycyclic aromatic hydrocarbons apart from being persistent in the environment are also toxigenic, carcinogenic and mutagenic (Mrayyana and Battikhi, 2005). Polycyclic aromatic hydrocarbos (PAH) are produced during fuel combustion, waste incineration or as by-products of Industrial processes such as coal gasification, production of aluminum, iron, steel and petroleum refining (Fagade, 1990; Igwo-Ezikpe*et al*., 2010). Incessant discharge of chemically treated petroleum refinery effluent into contiguous host communities as well as neighbouring water bodies pose a threat to the microbiota, fauna, flora and overall health condition of humans due to the movement of toxic chemicals through the food chain. Hence, there is the need for an environmentally safe and cost effective approach for the removal of toxic materials (especially the polycyclic aromatic hydrocarbon fraction) from untreated effluent prior to discharge. Although several works have been done on biodegradation of refinery effluent (Okerentugba and Ezeronye, 2003; Idise*et al*., 2010), as well as degradation of polycyclic aromatic hydrocarbons in their pure form (Igwo-Ezikpe*et al*., 2010), studies have not been conducted to ascertain bacterial species preference for the polycyclic aromatic hydrocarbons contained in the effluent and this has necessitated this study.

2. Materials and Methods

2.1. Sample Collection

With the aid of a hand trowel, about 500g of soil samples were collected in sterile polythene bags at two different locations within the refinery jetty and designated A and B respectively. Untreated refinery effluent was collected in a sterile container from a holding tank within the refinery while 1.5 litres of water sample was collected in a sterile container from Ekpan River in Ekpan community, Warri Delta State. Samples were transported in ice-packs to the Environmental and Biotechnology laboratory in the Department of Microbiology, University of Ibadan and were stored in the refrigerator at 4°C prior to physico-chemical and microbiological analysis.

MEASUREMENT OF PHYSICO-CHEMICAL PARAMETERS

Determination of Nitrogen: The total Nitrogen in the soil samples was determined using the regular Macro-Kjeldahl method (Keeney and Nelson, 1982).

Determination of Phosphorus: The phosphorus content of the soil samples was determined by the colorimetric method of Murphy and Riley (1962).

Determination of Soil pH: Soil pH was determined by the glass electrode pH meter (Adwa pH-Adwa Microprocessor pH meter) using soil and water ratio 1:1.

Determination of Available Potassium: Two grams of 2mm sieved soil was weighed into an extraction cup and 20 ml of ammonium acetate added at a pH of 7 and was agitated using a mechanical shaker for about 20mins and filtered using a 9mm filter paper. The filtrate obtained was read using an atomic absorption spectrophotometer (BUCK Scientific 2010/2011 VGP).

Determination of Soil Conductivity: Ten grams of 2mm sieved soil was weighed into an extraction cup and 10 ml of distilled water was added and the resulting mixture was agitated on a mechanical shaker. The conductivity was determined using a conductivity meter (Hama Instruments DIST Conductivity/TDS meters S399590).

Determination of Hydrocarbon content: The total hydrocarbon content of soil samples was determined by gravimetric method.

MICROBIOLOGICAL ANALYSIS

Enumeration of Total Heterotrophic bacteria: The total heterotrophic bacteria count was determined on nutrient agar using pour plate method.

Enumeration of Total Culturable Hydrocarbon-Utilizing bacteria: The total culturable hydrocarbon-utilizing bacteria was enumerated on mineral salts agar (Mills *et al*., 1978) using the spread plate method. The vapour phase transfer method of Raymonds*et al*. (1976) was used for this study.

SCREENING FOR BIOSURFACTANT PRODUCTION

The hydrocarbon-utilizers were screened for biosurfactant production using the mineral salts medium described by Salehizadeh*et al*. (2009).

Emulsification activity (Emulsification test (E24)): The ability of the biosurfactant to emulsify crude oil was determined following the method described by Salehizadeh*et al*. (2009). The crude biosurfactant was obtained by centrifuging the production broth at 8000 rpm for 15mins so as to separate the cells from the supernatant. 2 ml of crude oil was suspended in test tubes containing 2 ml of cell-free supernatant. The mixture was vortexed at high speed using a vortex mixer (model 1291) for 2mins and the test tubes were left to stand for 24h after which the emulsification index was measured. The emulsification index (E24) is given as the percentage of the height of the emulsified layer (cm) divided by the total height of the liquid column (cm) and multiplied by 100 (Ilori*et al*., 2005; Salehizadeh*et al*., 2009).

SCREENING FOR PAH DEGRADERS

Hydrocarbon-utilizing bacteria were screened for their ability to utilize PAH as sole carbon and energy source on Bushnell-Haas medium fortified with 2% agar. Low molecular weight PAHs (anthracene and phenanthrene) were used for this study. The sublimation method described by Alley and Brown (2000) was employed with some modifications.

IDENTIFICATION OF PAH-DEGRADERS

Identification of PAH-degraders was carried out on the basis of morphology and biochemical reactions.

BIODEGRADATION OF PAH-FRACTION OF REFINERY EFFLUENT

The biodegradation study was conducted using the method described by Okerentugba and Ezeronye (2003) with some modifications. Bushnell-Haas medium was dispensed into 100 ml Erlenmeyer flasks. To each flask was added 5% (v/v) of refinery effluent so that the medium amounted to 99 ml. Test isolates were sub cultured on nutrient agar and incubated at a temperature of 28±2^{o}C for 24h after which they were harvested in sterile distilled water. Flasks were sterilized and inoculated with 1.0ml suspension of test isolates in sterile distilled water. The initial absorbance of the inoculum was measured at 540 nm on a UV-visible spectrophotometer (Camp sec M105) and standardized at 0.8 A^{o} for the isolates singly and the mixed bacterial culture respectively. The flasks were incubated at room temperature with shaking on a rotary shaker incubator at 160 rpm for 15 days. The set-up was arranged in 5 replications with each flask sacrificed every 72hours during the extraction of degraded effluent. The optical density of the culture medium was determined at intervals of 72hours using a visible spectrophotometer at 600nm.

Extraction of degraded effluent was carried out using n-hexane and methanol in the ratio 3:1. An equal volume of culture was added to the solvent in a separating funnel, agitated and allowed to stand so as to separate the medium from the degraded effluent. The degraded effluent was subjected to analysis using a Gas chromatography with flame Ionization detector.

3. Results and Discussion

3.1. Physico-chemical Properties of Soil Samples

The physico-chemical properties of the soil samples are shown in the Table 3.1 below:

Table 3.1

Properties	Soil sample A	Soil sample B
Organic carbon %	33.90	5.80
Total Nitrogen(g/kg)	3.52	0.60
pH	3.3	4.6
Temperature(°C)	28	28
Moisture content	96.08	95.53
Available phosphorus(g/kg)	26.50	17.80
Potassium(Cmol/kg)	0.059	0.094
Conductivity(µs/cm)	59.74	26.78
% TPH	36.28	0.67

Table 3.2. Physicochemical parameters of water sample

Water Sample	Parameter
pH	6.1
TDS(ppm)	40
Conductivity (µs)	80
Temperature(°C)	28.3
%TPH	0.58

Table 3.3. Total heterotrophic bacterial and hydrocarbon utilizers' count(cfu/ml) in samples

Sample	THC (10^6)	HYCUB	%HU
Soil (A)	0.6	2.0	33.3
Soil (B)	2.7	1.4	0.05
Water	3.2	1.0	0.03

The higher population of heterotrophic bacteria in soil sample B in comparison with soil sample A could be attributed to the percentage of total petroleum hydrocarbon. This agrees with findings of several authors who reported that there is always a higher prevalence of heterotrophs as well as a diverse bacterial population in a sample that is less polluted with hydrocarbons compared with one that is more polluted (Amund and Igiri, 1990; Akpor*et al*., 2007). The occurrence of hydrocarbon utilizers among the heterotrophic population in the samples is an indication of previous contamination due to hydrocarbon pollution (Lewis *et al*., 1984; Adelowo*et al*., 2006; Singh and Lin, 2008; Al-thani*et al*., 2009).

Out of the 41 hydrocarbon utilizers obtained from the samples only 26(63.4%) gave a positive result for biosurfactant production when a mixture of their cell-free supernatant and crude oil was used for emulsification assay. The highest percentage emulsification was produced by isolate ER4 (67%) while isolate ER1 had the lowest with 7%. The occurrence of biosurfactant producers in a hydrocarbon polluted environment could be attributed to the role of bio surfactants in hydrocarbon degradation by bacteria through solubilization, mobilization and emulsification of the hydrocarbons, thus, allowing for their bioavailabity (Deziel, 1996; Adelowo and Oloke, 2002; Nweke and Okpokwalisi 2003; Adelowo*et al*., 2006; Adebusoye*et al*., 2008; Balogun and Fagade, 2010).

Table 3.4. Assay of the emulsification capacity of biosurfactant producing isolates

BacterialIsolates	PercentageEmulsification (%)
SA	48
SA1	40
SA2	42
SA4	43
SA6	52
SA7	57
SA8	35
SA9	34.8
SA10	37
SB1	44
SB2	11
SB3	33
SB4	23
SB5	33
SB7	40
SB8	42
SB9	38
SB10	56
SB11	49
ER	23
ER1	7
ER3	40
ER4	67
ER5	22
ER7	56
ER8	29

Key: SA – Soilsample A; SB – Soilsample B; ER – Ekpan River sample.

3.2. Screening for PAH-degraders

A total of 3/41(7.32%) isolates were capable of utilizing phenanthrene and Anthracene as sole carbon and energy sources which was indicated as a zone of clearing around their colnies. These isolates were characterized and identified by making reference to Bergey's Manual of Determinative bacteriology and Cowan and Steel's Manual of Medical Microbiology. These PAH-degraders belonged to the genera *Pseudomonas* and *Achromobacter* respectively.

Table 3.5. Biochemical characterization of PAH-degraders

Identification characters	Isolate		
	SB	SB1	SB3
Gram reaction	-	-	-
Shape	Short rods	Short rods	Short rods
Catalase	+	-	+
Oxidase	+	+	+
Nitrate	+	+	+
Urea hydrolysis	+	+	+
Motility	+	-	+
Methyl-Red	-	-	-
Voges-Proskauer	-	-	-
Simmon's Citrate	+	+	+
Casein hydrolysis	-	-	-
Glucose	-	+	-
Arabinose	-	-	-
Fructose	-	d	-
Maltose	-	-	-
Mannitol	-	-	-
Lactose	-	-	-
Sucrose	+	-	+
Starch hydrolysis	+	-	+
Growth on Cetrimide	+	+	+
Gelatin hydrolysis	-	-	-
Probable Identity	*Pseudomonaas*sp	*Achromobacterxylosoxidans*	*Pseudomonas*sp

Key: + = positive, - = negative, d = different reactions given by different strains, positive test often delayed.

PAH-degraders were characterized and identified as *Pseudomonas*sp (SB), *Achromobacterxylosoxidans* (SB1) and *Pseudomonas*sp (SB3) using various morphological and biochemical tests according to Bergey's Manual of

Systematic Bacteriology 9th Edition (Holt *et al*., 1994) and Cowan and Steel's Manual for Identification of Medical bacteria (3rd Edition) (Barrow and Feltham, 1993).

The occurrence of only 3 PAH-utilizers which represents 7.32% (3/41) of the hydrocarbon utilizers indicates that only these isolates had the physiological capabilities to metabolize the aromatic hydrocarbons (Amund and Igiri, 1990; Woo *et al*., 2004; Santos *et al*., 2008; Hassan *etal*., 2009). The ability of *Pseudomonas*sp to metabolize low molecular aromatic polycyclic hydrocarbons such as naphthalene, anthracene and phenanthrene as well as high molecular PAHs such as chrysene, pyrenee.t.c has been reported (Meyer *et al*., 1999; Woo *et al*., 2004; Santos *et al*., 2008; John *et al*., 2012). *Achromobacterxylosoxidans* has also been reported to be capable of metabolizing PAH (Wan *et al*., 2006; Al-thani *et al*., 2009).

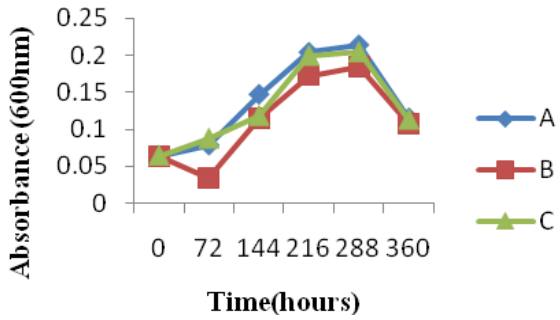

Figure 3.1. Growth of the cultures on biodegradation medium

Key
A – *Pseudomonas*sp SB
B – *Achromobacterxylosoxidans* SB1
C – *Pseudomonas*sp SB + *Achromobacterxylosoxidans* SB1

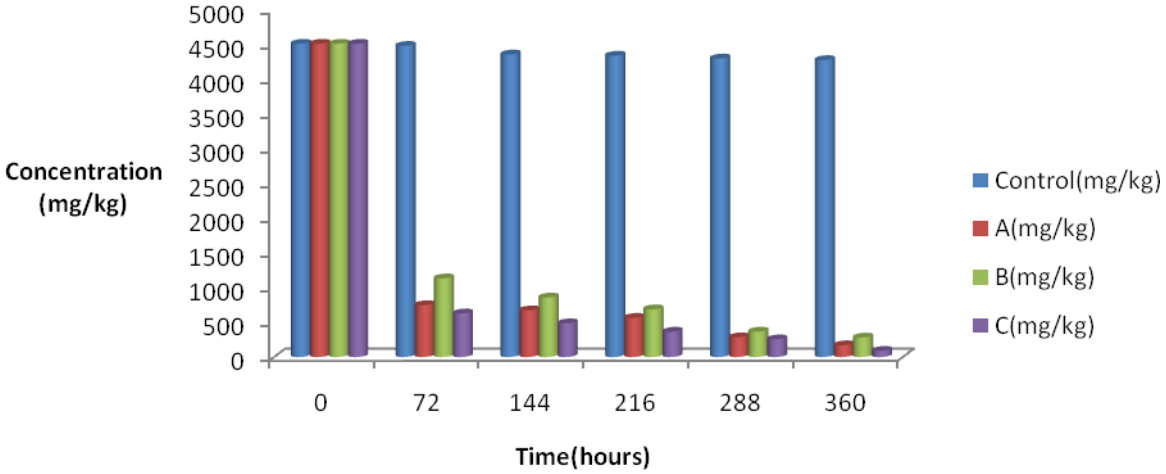

Figure 3.2. Reduction in the concentration of PAH in Refinery Effluent

Key
A – *Pseudomonas*sp SB
B – *Achromobacterxylosoxidans* SB1
C – Mixed culture
CTRL – Control.

4. Conclusion

From this study it can be inferred that the prevalence of heterotrophs in an environment is proportional to the extent of hydrocarbon pollution. It is also pertinent to note that not all heterotrophs are capable of utilizing hydrocarbons hence the population of hydrocarbon utilizers is always lower when compared to the heterotrophic population and this may be attributed to the fact that not all bacterial species are hydrocarbon tolerant let alone capable of utilizing hydrocarbons as sole carbon and energy source during growth.

Although biosurfactant production is important for hydrocarbon metabolism by bacteria, not all hydrocarbon utilizers give a positive result for emulsification capacity assay. This is because biosurfactants produced by microorganisms in the course of their growth and metabolism either solubilize, mobilize or emulsify the hydrocarbon substrate and as such are grouped on the basis of their functions. It is on this basis that we can say that all emulsifiers are surfactants but not all surfactants are emulsifiers. From this research work, it can be inferred that although not all hydrocarbon utilizers are capable of producing emulsifiers, but then there is always a tendency for most hydrocarbon utilizers to be able to emulsify hydrocarbon substrates for metabolism.

This study has also shown that not all hydrocarbon utilizers obtained from an environment are capable of utilizing polycyclic aromatic hydrocarbons even though they may have been isolated from a hydrocarbon polluted environment. The use of crude oil as sole carbon and energy source for the isolation of hydrocarbon utilizers does not necessarily confer the ability of the isolates obtained to be able to metabolize the aromatic fraction. This may be due to the fact that most bacteria isolates select only hydrocarbons they can easily metabolize which in most cases constitute the non-recalcitrant fraction and as such are unable to thrive in a medium comprising of recalcitrant hydrocarbons such as polycyclic aromatic hydrocarbons as sole carbon and energy source.

Biosurfactant production has been reported by several authors as being very important in bacterial degradation of polycyclic aromatic hydrocarbons owing to the fact that it ensures bioavailability of the hydrocarbons, thus promoting degradation. However, the bioavailability of a substrate is just part of the requirements for its metabolism as there is also the need for the organism in question to

have the necessary physiological capabilities in place to allow for metabolism.

Polycyclic aromatic hydrocarbons have been reported to be toxic to microorganisms. Mobilization of these compounds into cells of organisms would only give rise to cytotoxic effects and would cause harm to the organism instead of fostering degradation. This study has revealed that not all hydrocarbon utilizers are capable of metabolizing polycyclic aromatic hydrocarbons and this may be due in part to the absence of the necessary physiology for the metabolism of this group of hydrocarbons.

The ability of the bacterial isolates (*Pseudomonas* sp (SB) and *Achromobacterxylosoxidans* (SB1) employed in this study to degrade the polycyclic aromatic hydrocarbon fraction of the refinery effluent indicates that microorganisms capable of degrading certain hydrocarbon pollutants in the pure form are also capable of degrading such pollutants when found in a mixture with other pollutants.

References

[1] Adebusoye, S.A., Amund, O.O., Ilori, M.A., Domeih, D.O. and Okpuzor, J. 2008.Growth and biosurfactant synthesis by Nigerian hydrocarbon-degrading estuarine bacteria. International *Journal of Tropical Biology*, 56:4:1603-1611.

[2] Adelowo, O. O and Oloke, J.K. 2002.Comparison of the Emulsification and Oil Displacement Profile of some Biosurfactants. *NISEB J*. 2. 3:181-186.

[3] Adelowo,O.O., Alagbe, S.O. and Ayandele, A.A. 2006.Time-dependent stability of used engine oil degradation by cultures of *Pseudomonas fragi*and *Achromobacteraerogenes*. *African Journal of Biotechnology*. 5. 24:2476-2479.

[4] Akpor O.B., O.E Igbinosa and O.O Igbinosa, 2007.Studies on the Effect of Petroleum Hydrocarbon on the Microbial and Physico-Chemicals Characteristics of Soil.*African Journal ofBiotechnology*. 6. 16: 1939-1943.

[5] Alley J.F and L. R. Brown. 2000. Use of Sublimation to Prepare Solid Microbial Media with Water-Insoluble Substrates. *Applied and Environmental Microbiology*. 66.1: 439-442.

[6] Al-Thani, R.F., Abd-El-Haleem, D.A.M. and Al-Shammri, M. 2009.Isolation and characterization of polyaromatic hydrocarbons-degrading bacteria from different Qatari soils.*African Journal of Microbiology Research*.3.11: 761-766.

[7] Amund O. O andIgiri C. O. 1990.Biodegradation of petroleum hydrocarbon under tropical estuarine conditions.*World Journal of Microbiology and Biotechnology*.16: 118-121.

[8] Balogun, S.A. andFagade, O.E. 2010.Emulsifying bacteria in produce water from Niger-Delta, Nigeria.*African Journal of Microbiology Research*. 4. 9:730-734.

[9] Barrow, G. I. and Feltham, R. K. A. 1993. Cowan and Steel's Manual for the Identification of Medical Bacteria, Cambridge University press.

[10] Déziel, E., Paquette, G., Villemur, R., Lépine, F. and Bisaillon, J. 1996. Biosurfactant production by a soil *Pseudomonas* strain growing on polycyclic aromatic hydrocarbons. *Applied Environmental Microbiology*.62:1908-12.

[11] Fagade, O. E. 1990. Bacterial Degradation of Crude oil from the Niger Delta area of Nigeria. Unpublished Ph.D Thesis, Dept of Botany and Microbiology University of Ibadan, Nigeria. pp. 77-79.

[12] Harry, M.F. 1995. Industrial Pollution Handbook. McGraw Hill. Inc., New York

[13] Hassan E. A., Elsayed E. H., Azhar A. H., Amany, G. A. and Amr A. E. 2009. Isolation and Identification of Three-RingsPolyaromatic Hydrocarbons (Anthracene and Phenanthrene) Degrading Bacteria. *American-Eurasian Journal of Agriculture and Environmental Science*.5.1: 31-38.

[14] Holt, J.G., Kreig, W.R., Sneath, P.H.A., Staley, J.T and William, S.T. 1994.Bergey's Manual of Determinative Bacteriology, 9thedn, Williams and Wilkinson, A., Waverly Company.

[15] Idise, O. E., Ameh, J.B., Yakubu, S.E. andOkuofu, C.A. 2010. Biodegradation of a refinery effluent treated with organic fertilizer by modified strains of *Bacillus cereus* and *Pseudomonas aeruginosa*.*African Journal of Biotechnology*, 9. 22: 3298-3302.

[16] Igwo-Ezikpe, M.N., Gbenle, O.G., Ilori, M.O., Okpuzor, J. andOsuntoki, A. A. 2010. High Molecular Weight Polycyclic Aromatic Hydrocarbons Biodegradation by Bacteria Isolated from Isolated from Contaminated Soils in Nigeria. *Research Journal of Environmental Sciences.* 4.2: 127-137.

[17] Ilori, M.O., Amobi, C.J. and Odocha, A.C. 2005. Factors affecting biosurfactant production by oil degrading *Aeromonas* sp. isolated from a tropical environment. *Chemosphere* 61:985-992.

[18] John R. C., Essien, J. P., Akpan,S. B. and Okpokwasili, G. C. 2012. Polycyclic Aromatic Hydrocarbon-degrading Bacteria from Aviation Fuel Spill Site at Ibeno, Nigeria.*Bull Journal of Environmental Contamination and Toxicology.* 88:1014-1019.

[19] Keeney, D. R. and Nelson, D. W. 1982. Nitrogen-Inorganic forms. In: Methods of Soil Analysis part 2: Chemical and microbiological properties, 2nd edn, page AL, Miller R. H, Keeney D.R.(eds) Agronomy 9/2, American society of Agronomy, Madison, WI, pp. 643-698.

[20] Lewis, D., Hodson, R. and Freeman, L. 1984. Effects of microbial community interactions on transformation rates of xenobioticchemicals.*Applied Environmental Microbiology* 48: 561-565.

[21] Mills, A. L., Breuil, C. and Colwell. 1978. Enumeration of Petroleum degrading marine and estuarine microorganisms by most probable number method. *Canadian Journal of Microbiology*, 12: 234-248.

[22] Mrayyana, B. andBattikhi, M.N. 2005.Biodegradation of total organic carbons (TOC) in Jordanian petroleum sludge.*Journal of HazardousMaterial*. 120: 127-134.

[23] Murphy, J. and Riley, J.P. 1962. Analytical Chemistry Acta. 2. 27: 31-36.

[24] Nweke, C. O. and Okpokwasili, G. C. 2003. Drilling Fluid Base Oil Biodegradation Potential of a Soil *Staphylococcus* Species.*African Journal of Biotechnology*. 2.9: 293-295.

[25] Okerentugba, P. O. and Ezeronye, O. U. 2003. Petroleum Degrading Potentials of Single and Mixed Microbial Cultures Isolated From Rivers AndRefinery Effluents In Nigeria. *African Journal of Biotechnology*. 2.9: 293-295.

[26] Raymond, R.L., Audson, J. O. and Jamison, V.W. 1976. Oil degradation insoil. *Applied Environmental Microbiology*. 31: 522–535.

[27] Saien, J. andNejati, H. 2007. Enhanced photocatalytic degradation of pollutants in petroleum refinery wastewater under mild conditions.*Journal of HazardousMaterial*. 148:491-495.

[28] Salehizadeh, H. and Mohammadizad, S. 2009. Microbial enhanced oil recovery using biosurfactant produced by *Alcaligenesfaecalis*. *Iranian Journal of Biotechnology*.7.4: 216-223.

[29] Santos, E. C., Jacques, J. S. R., Bento, M. F., Peralba, R. M. C and Selbach, P. A *et al.* 2008. Anthracene biodegradation and surface activity by an iron-stimulated *Pseudomonas* sp. *Bioresource technology*, 2549-2644.

[30] Singh, C. and Lin, J. 2008. Isolation and characterization of diesel oil degrading indigenous microrganisms in Kwazulu-Natal, South Africa. *African Journal of Biotechnology*. 7.12: 1927-1932.

[31] Wan, N., Gu, J. and Yan, Y. 2006. Degradation of p-nitrophenol by*Achromobacterxylosoxidans* NS isolated from wetland sediment.*International journal of Biodeterioration and Biodegradation* 59: 90-96.

[32] Woo, S. H., Jeon, C. O. and Park, J. M. 2004. Phenanthrene biodegradation in soil slurry systems: Influence of salicyclate and triton X-100. *Korean Journal of Chemical Engineering*, 21: 412-418.